LESSON 8:
MERISTEM CULTURE

Objective

What is Meristem Culture?

Cultivation of axillary or apical shoot meristems, particularly of shoot apical meristem, is known as meristem culture. Meristem culture involves the development of an already existing shoot meristem and subsequently, the regeneration of adventitious roots from the developed shoots. It usually does not involve the regeneration of a new shoot meristem. The objective here, therefore, is to use shoot meristem of the plant as an explant and study the culture requirements and regeneration of the whole plant. Shoot tip culture finds a wide application in the production of virus-free plants which is discussed in the next Lesson in detail.

Explant Used

Shoot apical meristem lies in the 'shoot tip' beyond the youngest leaf or first leaf primordium (Fig.1). It measures up to about 100 um in diameter and 250 um in length. Thus a shoot tip of 100-500 um would contain 1-3 leaf primordia in addition to the apical meristem. In practice, shoot tips of up to 1 um are used when the objective is virus elimination. Shoot tip culture is widely used for rapid clonal propagation for which much larger, e.g., 5-10 mm explants are used. Therefore, most cases of meristem culture are essentially shoot-tip cultures. Nodal explants of various sizes are also commonly employed for rapid clonal propagation.

When the objective is vegetative propagation, the size of shoot tip used for culture is not important. But when the objective is to free the stock from a virus, it is essential that the apical meristem should be excised along with a minimum of the surrounding tissue. The shoot tip may be cut into fine pieces to obtain more than one plantlet from each shoot-tip. In species like cauliflower, pieces of curd (the inflorescence) are used, while in plants having underground stems shoot tips or tissue pieces bearing buds of such stems may be used. Generally, explants taken from actively growing plants at the beginning of growing season are the most suitable.

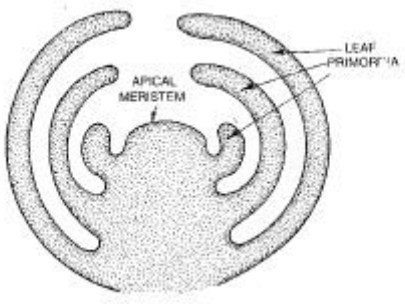

Fig. 1. A longitudinal section through a shoot tip; shoot apical meristem plus the first three leaf primordia are shown.

Culture Medium

In general, MS medium has been found satisfactory for most plant species. But for some species a much lower salt concentration may be adequate or even necessary, since the high salt concentration of MS medium may be deleterious or even toxic. For example for blueberry, 1/4 MS salts are the best, while full MS is often toxic. Agar gelled medium is the most widely used mainly for convenience. But in some species, use of liquid medium is either necessary, e.g., Cattleya, or beneficial, e.g., Cephalotus.

Growth Regulators

The GR requirement depends on the stage of culture process:

i. culture initiation,

ii. shoot multiplication,

iii. rooting of shoots and

iv. transfer of plantlets to pots/soil

Culture initiation consists of surface sterilization of explants and establishing them in vitro. The main feature of this stage is detection and elimination/ control of contamination, growth of explants may or may not occur. Generally, a GR-free basal medium is used. In cases of heavy contamination or endophytic contamination (bacteria/fungi present inside explant) a suitable antibiotic, e.g., trimithoprim, and/or fungicide, e.g., Bavistin, may be added to the culture medium.

After 2-3 weeks, the cultures are transferred to a shoot multiplication medium designed to promote axillary branching. This medium generally contains a cytokinin (usually 1-2 mg/l, but up to 30 mg/l has been used) either alone or in combination with an auxin (commonly 0.1-1 mg/l), chiefly depending on the plant species. BAP is the most commonly used cytokinin, but with some species, e.g., blueberry, garlic, rhododendrons etc., 2-ip is much more effective. Among auxins, NAA, IBA and IAA are generally employed. 2,4-D is not used as it promotes callusing. Higher concentrations (>2 mg/l BAP) of cytokinin induce adventitious buds and retard shoot growth. The latter may necessitate a culture of shoots on basal/low cytokinin/ GA3 medium for shoot elongation before they can be rooted. Therefore, a GR combination should be determined to obtain optimum shoot multiplication rates with the minimum risk of adventitious shoot buds and, if possible, without the need of shoot elongation step (to save time, labour and cost).

Environment during Culture

During culture initiation and shoot multiplication phases, the cultures are generally kept at a constant temperature of 25°C and are illuminated with about 1000 lux white light from fluorescent tubes. In some cases, a high light intensity, e.g., 3000 lux in Gerbera, may have detrimental effects. But during rooting, higher light intensities, e.g., 3,000-10,000 lux are commonly used since there is a beneficial effect on rooting and on plant survival on transfer to soil.

Browning of Medium

In many species, phenolics leach into the medium from the cut surfaces of explants. These phenolics turn dark brown on oxidation and are detrimental to the cultures. This problem is very common in case of woody species, particularly when explants are taken from mature trees. This problem can be overcome, in most species, in one of the following ways, but in some species like mango control of phenolics is the chief problem since the entire explant turns black and dies.

1. Frequent (every 3-7 days) subculture of explants on agar medium may be sufficient to overcome this problem in many species.
2. A brief period (usually 3-10 days) of culture in liquid medium is effective in many species, e.g., apples, Rubus, Eucalyptus etc. This may remove from the explants not only polyphenols but other possibly inhibitory substances.
3. In difficult cases, an antioxidant like ascorbic acid (50-100 mg/l), cysteine-HCI (100 mg/l) or citric acid (150 mg/l) may be used to check the oxidation of polyphenols.
4. Adsorbents like activated charcoal (0.5-2 g/l) or PVP (polyvinyl-pyrrolidon) may be used to adsorb the polyphenols secreted into the medium.
5. Culture in dark may be helpful since light enhances polyphenol oxidation.

Rooting of Shoots

In general, the rooting medium has low salt, e.g., 1/2 or even 1/4 salts of the MS medium, and reduced sugar levels (usually 1g/l), reduced salts being essential for rooting in some species like Narcissus. In some species, e.g., Narcissus, strawberry etc., rooting occurs on GR-free medium. But in most species, 0.1-1 mg/l NAA or IBA is required for rooting. In plants like Citrus, however, a pulse treatment with an auxin (10 min with 100 mg/l NAA or IBA) gives optimum rooting.

Shoots are usually rooted in an agar medium, but the recent trend is to root them directly in vermiculite or potting mix. The cut ends of shoots are treated with a suitable auxin solution or powder mix, transplanted in pots and kept under high relative humidity and low light intensity. This saves cost as rooting and soil transfer stages are combined and the rooting medium is eliminated. Rooting takes about 10-15 days, depending mainly on species. Plantlets with 0.5 to 1 cm roots are usually transplanted into pots since longer roots tend to get damaged.

Transfer of Plantlets to Soil

Rooted shoots are removed from the medium, agar sticking to their roots is washed with tap water, and they are transplanted into plastic cups containing a suitable potting mix. Plants are kept in a high (90%) humidity and, initially low light intensities. High humidity can be attained by:

i. fog (water drops 10um or less)

ii. mist, or

iii. a clear plastic to cover individual (plastic bags) or groups (plastic sheets) of plants.

The potting mix should not be too wet and water drops should not form on the plantlets.

For this reason, fog is preferred over mist. The humidity is gradually decreased to the ambient level after about 7- 15 days, and the light intensity is increased. The plants are finally exposed to greenhouse conditions. On a laboratory scale, individual plants may be covered with clear plastic bags and irrigated daily with 2-3 drops of water or ¼ MS salts. After 7-10 days, the bags may be removed gradually.

Vitrification and Morphological Variations

What is Vitrification?

Some shoots developed in vitro appear brittle, glassy and water soaked this is called vitrification. In many species, vitrification may be represented by symptoms not visible to the naked eye, e.g., poorly developed vascular bundles, abnormal wax quality, abnormal functioning stomata etc. Vitrification is the consequence of culture conditions, and leads to losses of plantlets. It may be overcome by the following:

i. increased agar levels (1%),

ii. bottom cooling of culture vessels,

iii. addition of agar hydrolysates, and

iv. use of growth retardants.

Morphological variants may arise during the multiplication stage. Such variants occur, in most cases, at a frequency comparable to that in vivo. But in commercial ventures using meristem cultures visual selection is practised to eliminate variants and maintain the homogeneity of plantlets produced. Many commercial enterprises, therefore, prefer to multiply shoots for only four or so cycles from an explant; after this a fresh batch of cultures is initiated from field-tested plants.

Conclusion

After understanding the methods of single cells culture, callus and suspension cultures, this lesson gives an idea about what type of explants can be used in tissue culture systems. Explants are selected in a way that they have some practical application. For Example, shoot meristem culture has been widely employed in producing virus- free plants. The size of the explant is also critical for virus elimination like, shoot tips are used here. This Lesson gives the basic requirements for culture of meristems and further regeneration of whole plants and the practical applications of this type of culture is given in the next Lesson.

Questions

1. What type of explant will you use to initiate shoot meristem culture? Explain why.
2. Discuss the cultural requirements (from initiation of cultures to transfer of plants to soil).
3. Write short notes on:
   a. Vitrification
   b. Browning of medium